

Figure Legends

Figure 1: Immunostaining for p-mTOR.

A: Oesophageal squamous cell cancer cells positive for p-mTOR (white arrow).

B: Oesophageal squamous cell cancer cells negative for p-mTOR.

C: Western blot analysis of mTOR, p-mTOR, and β -actin levels in TE1, 4, 9, 11, and 13 cell lines.

Figure 2: Western blot analysis for p70S6k, p-p70S6k, 4E-BP1, and p-4E-BP1 protein levels in TE4 and TE11 cells treated with (at indicated concentrations) or without everolimus

Figure 3: *In vitro* assay for confirming the anti-cancer activity of everolimus.

A: *In vitro* proliferation assay. Treatment with everolimus (20 nM) for 48 h decreased the proliferation ratios of both TE4 and TE11 cells compared with those of control vehicle-treated cells. *, $P < 0.05$

B: *In vitro* cell cycle assay. Treatment with everolimus (20 nM) increased the percentages of TE4 and TE11 cells in G_0/G_1 phase compared with those of control vehicle-treated cells. *, $P < 0.05$

C: *In vitro* cell apoptosis analysis. Induction of early apoptosis in TE4 and TE11 cells by everolimus is shown (lower right part; Annexin V-FITC-positive, PI-negative).

D: *In vitro* invasion assay. Everolimus (20 nM) decreased the numbers of invading TE4 and TE11 cells compared with those of control vehicle-treated cells

(200× magnification, 5 fields). *, $P < 0.05$

Figure 4: *In vivo* assay for confirming the anti-cancer activity of everolimus utilizing a mouse xenograft model established with TE4 cells.

A: Treatment schedules for the 4 treatment groups (placebo, everolimus, cisplatin, and everolimus plus cisplatin).

B: Tumour volume in the 4 treatment groups (placebo, everolimus, cisplatin, and everolimus plus cisplatin) after the 5-week course of treatment.

C: Growth of tumour volume in the 4 treatment groups.

Supplemental

Supplemental Figure 1

Western blot analysis for Bad and PERP in TE4 cells treated with everolimus (20nM).

Supplemental Figure 2: *In vivo* assay for confirming the anti-cancer activity of everolimus utilizing a mouse xenograft model established with TE11 cells.

A: Tumour volume in the 4 treatment groups (placebo, everolimus, cisplatin, and everolimus plus cisplatin) after the 5-week course of treatment.

B: Growth of tumour volume in the 4 treatment groups.

Supplemental Figure 3

The weight changes of the mice in the 4 treatment groups (placebo, everolimus,

cisplatin, and everolimus plus cisplatin) during the 5-week course of treatment. The mean day-36 weights of mice treated with placebo, everolimus, cisplatin, and everolimus plus cisplatin were $19.8 \pm 0.83 \text{ mm}^3$, $21.9 \pm 1.78 \text{ mm}^3$, $21.6 \pm 1.35 \text{ mm}^3$, and $21.8 \pm 0.93 \text{ mm}^3$, respectively. There was no significant difference among the 4 groups.

Supplemental Figure 4

Histological evaluation of organ injury (**A, F, K, P**: liver, **B, G, L, Q**: pancreas, **C, H, M, R**: kidney, **D, I, N, S**: lung, and **E, J, O, T**: intestine) in the mice in the 4 treatment groups (**A–E**: placebo, **F–J**: everolimus, **K–O**: cisplatin, **P–T**: everolimus plus cisplatin).